High-normal alanine aminotransferase as a Predictor of significant liver histological injury in Chronic Hepatitis B Virus Infection with different HBV DNA levels

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Abstract

Objective

We aimed to assess significant liver histological injury (SLHI) of chronic hepatitis B (CHB) patients with normal ALT in order to select clinical threshold of ALT.

Methods

We retrospectively examined 347 patients who underwent liver biopsy in Beijing You an hospital, Capital Medical University from January 2018 to January 2021. Univariate analysis of the clinical parameters versus inflammation and fibrosis was carried out.

Results

Of these 347 patients met the inclusion criteria, 174 (50.1%) patients had SLHI totally. The AUROC of the ALT associated with the diagnosis of SLHI was 0.572 for female and 0.6 for male with a cut-off value of 20 U/L and 25 U/L, respectively ($p = 0.04$, $p = 0.013$). Among HBeAg-positive patients, the proportion of SLHI in the high-normal ALT group (ALT > 20 U/L for female, > 25 U/L for male) with low HBV DNA loads ($< 7 \log_{10}$ IU/ml) was higher (66.7% vs 33.3%, $p < 0.001$); also the proportion in the HBeAg-negative with high-normal ALT and HBV DNA ≥ 2000 IU/ml was significantly higher (70% vs 37.7%, $p < 0.001$).

Conclusion

Clinical threshold of ALT to distinguish SLHI was 25 U/L for male and 20 U/L for female. The CHB patients with high-normal ALT and certain HBV DNA level (≥ 2000 IU/ml for HBeAg-negative and $< 7 \log_{10}$ IU/ml for HBeAg-positive) were suggested to perform liver biopsy or noninvasive methods for histopathology assessment, then to be determined for antiviral therapy.

Introduction

Chronic hepatitis B virus (HBV) infection is a public health problem worldwide, and may cause a spectrum of diseases ranging from asymptomatic state to cirrhosis and hepatocellular carcinoma (HCC) [1]. Among chronic HBV infection patients, those with significant liver histological injury (SLHI) defined as inflammatory grade $\geq G2$ and/or fibrosis stage $\geq S2$ using the METAVIR scoring system are at increased risk for developing cirrhosis and HCC and should be considered for antiviral therapy immediately [2]. Liver biopsy is the gold standard for the diagnosis of SLHI [3], but it examines only a very small part of the liver and there is a risk that this part might not be representative for the whole liver. Alanine transaminase (ALT) is the least costly and most widely used marker in chronic HBV infection patients [4]. Current guidelines on the management of chronic hepatitis B (CHB) suggest that ALT more than upper limit of normal (ULN) should be one of the indications for antiviral therapy, and patients with normal ALT levels are considered to have "healthy" livers and may be suggested for regular follow-up rather than antiviral therapy immediately [2,5-6].

However, the definition of ULN for ALT in different laboratories and CHB guidelines is not consistent. Current ULN for ALT level were set, on average, at 40 U/L in studies conducted over the past 10 years [7-9]. Such threshold was adopted by EASL and APASL as a crucial criterion for determining treatment initiation for CHB. As for AASLD, the ULN of 35 U/L ALT for male and 25 U/L for female adults is recommended when guiding management decisions, which can improve disease outcome [5]. The selection of these cut off levels is based on the findings presented above in which patients with ALT levels above these cut-off values had a significantly higher rate of advanced fibrosis but below which minimal histologic adversity was observed [10-13].

Compared with the threshold of ALT reduction in Europe and the United States, CHB guideline in China [6] requires ALT level $> 1 \times$ ULN as major determinant for indicating treatment initiation, which are based on ALT norms, expressed as a multiple of the ULN, defined by local laboratories; in other words, no absolute levels have been mentioned. In our clinical practice, normal ALT level was defined as 9 ~ 50 U/L for male, 7 ~ 40 U/L for women which was based on the industry standard issued by the Ministry of Health in 2013 [14]. Obviously, unlike the range of normal ALT, clinical threshold of ALT is based on a special group infected with chronic HBV, where ALT levels represent significant inflammation and fibrosis of the liver and requires antiviral therapy, based on the predictive efficacy of ALT for disease prognosis. So in this study, we aim at comprehensively evaluating the characteristics of histological abnormalities by a large population of Chinese CHB patients with normal ALT, selecting clinical threshold of ALT to distinguish significantly higher rate of SLHI.

Methods

Study population

A total of 768 consecutive chronic HBV infection patients who underwent liver biopsies and routine laboratory tests at Beijing Youan hospital, Capital Medical University between January 2018 and January 2021 were retrospectively screened for inclusion in the study. Chronic HBV infection was defined as the persistent presence of serum HBV surface antigen (HBsAg) for more than 6 months^[6]. The included subjects were treatment-naïve patients with PNALT levels defined as normal ALT (ULN: Male ≤ 50 U/L; Female ≤ 40 IU/L) measured on at least three occasions at intervals of more than 2 months apart over a period of 12 or more months before liver biopsy. Patients with the following conditions were excluded from this study: significant alcohol consumption (>20 g/day) (47 patients); accompanied by non alcoholic fatty liver disease (99 patients); accompanied by autoimmune liver disease (27 patients); co-infection with hepatitis C virus, hepatitis D virus, or human

immunodeficiency virus (48 patients); prior or current antiviral treatment (52 patients); and ALT $>1 \times$ ULN (148 patients). Finally, as shown in **Fig.1**, 347 treatment-naïve chronic HBV infection patients with PNALT levels were included. The study protocol was permitted by the ethics committee of Beijing Youan hospital, Capital Medical, and the procedures were in accordance with the Helsinki declaration of 1975, as revised in 1983 (The Code of Ethic Approval: [2020]089).

Liver histological examination

Ultrasonography-guided percutaneous liver biopsy was performed using a 16G

disposable needle (Hepafix, B. Braun, Melsungen, Germany) under local anesthesia.

Liver samples of minimum length 15 mm were immediately fixed with 10% formalin and embedded with paraffin. Liver tissue with at least six portal tracts was considered

sufficient for histological scoring. The METAVIR scoring system was adopted as the

diagnosis standard of liver inflammation and fibrosis according to the recommended standards of Chinese guidelines of the CHB prevention and treatment (2019 version)^[6]. Histological grading of liver inflammation is classified into G0 ~G4, and fibrosis was staged from F0 to F4. significant liver histological injury (SLHI) were defined as

inflammation grade ≥ 2 ($\geq$ G2) and/or fibrosis stage ≥ 2 ($\geq$ S2). All biopsy samples were interpreted independently by two liver pathologists. If they failed to reach an agreement, a third highly experienced pathologist reviewed the liver biopsy samples and the results were given by joint discussion of three pathologists.

Laboratory tests

Fasting blood samples were obtained, and routine laboratory tests were performed at the time of liver biopsies. The serological markers of HBV were detected with commercially available enzyme-linked immunosorbent assay (ELISA) kits (ARCHITECT i2000 SR; Abbott, Wiesbaden, Germany). The serum biochemical parameters including ALT, aspartate transaminase (AST), alkaline phosphatase (ALP), gamma-glutonyl transpeptidase (GGT), total bilirubin, albumin, and globulin were measured by full automation biochemistry analyzer (7600 Series; Hitachi, Tokyo, Japan). Routine blood test including white blood cell (WBC) count and platelet count was evaluated with an automated hematology analyzer (XT-2000i, Sysmex, Kobe, Japan). HBV DNA was quantified by the real-time PCR system (ABI 7500; Applied Biosystems, Foster City, CA, USA) with the lowest detection limit at 20 IU/ml.

Statistical analysis

Normality tests of all data were performed by Kolmogorov-Smirnov test. The baseline characteristics of enrolled patients are presented as follows: normal distribution data as mean $\pm$ standard deviation, non-normal distribution continuous data as median (interquartile range), and categorical variables as number (percentage). Chi-square test (for categorical variables), Mann-Whitney test (for non-normal distribution continuous variables), and t-test (for normal distribution variables) were performed to identify statistical differences between groups. Univariate and multiple logistic regression analyses were performed to identify the independent predictors of SLHI. All significance tests were two-tailed, and $p < 0.05$ was considered statistically significant. All statistical analyses were carried out using the SPSS statistical software version 23.0 (SPSS Inc., Chicago, Illinois, USA).

Results

Baseline characteristics of enrolled patients

A total of 347 chronic HBV infection patients were enrolled, and baseline characteristics were summarized in **Table 1**. The majority of enrolled patients were male (210, 60.5%), HBeAg positive (200, 57.64%) and middle-aged [38 (16-66) years]. Median HBV DNA, ALT, AST, ALP, GGT, albumin,

globulin, and PLT were 5.4 log₁₀ IU/ml (IQR=0-9.73), 26.3 U/L (IQR=5-50), 23.2 U/L (IQR=3-68), 70 U/L (IQR=59-85), 22 U/L (IQR=13-28), 43 g/L (IQR=41-47), and 206×10⁹/L (IQR=178-298), respectively.

Among 347 enrolled patients, 200 (57.64%) had HBeAg positive and 147 (42.36%) had HBeAg negative. The immune states of patients with CHB were defined according to Chinese 2019 Hepatitis B Guidance. The distribution of different immune states was as follows: Group A: Immune tolerant CHB (HBeAg positive, HBV DNA ≥10⁷ IU/ml), 108 (31.12%) patients; Group B: HBeAg-positive mild DNA load (HBV DNA 10⁴~10⁷ IU/ml), 72 (20.75%) patients; Group C: HBeAg-positive low DNA load (HBV DNA <10⁴ IU/ml), 20 (5.77%) patients; Group D: HBeAg-negative high DNA load (HBV DNA ≥2000 IU/ml), 86 (24.78%) patients; Group E: HBeAg-negative inactive (HBV DNA <2000 IU/ml), 61 (17.58%) patients.

There was no significant difference in sex, BMI, ALP, GGT, PLT among five groups. The HBeAg negative patients, especially for high HBV DNA load had significantly higher age (43.4 vs. 35-38 years, $p=0.001$). And a significantly lower ALT and AST were observed in HBeAg-negative inactive patients with lower HBV DNA load. (22.07 vs 26.7-28.6 U/L, $p<0.001$; 19.8 vs 22.1-27.4 U/L, $p<0.001$). Meanwhile, most of patients with HBeAg-positive and more than 10⁷ IU/ml HBV DNA load were male, compared with lower HBV DNA load (70.7% vs. 58.3%, $p=0.048$).

Prevalence of significant liver histopathology

The histopathological data of the liver biopsy samples are shown in Table 2. The majority of patients had minimal histological disease: 67.1% (Histological grading of liver inflammation G1) of the patients, while 29.1% of the patients had G2, 3.5% had G3, only 1 patient had G4, which 32.9% patients had significant inflammation (≥G2) totally. Regarding liver fibrosis, 26 patients were S0 (7.5%), 193 were S1 (55.6%), 84 were S2 (24.4%), 37 were S3 (10.7%), 7 were S4 (2%), which 36.9% patients had significant fibrosis (≥S2) totally. In addition, 174 patients had significant liver histological injury (SLHI) defined as ≥G2 and/or S2, the proportion was 50.1%.

The distributions of the inflammation and fibrosis stage among five groups are shown in Table 2 and Fig. 2. Significant differences in liver inflammation were observed in HBeAg-positive with less than 10⁷ IU/ml DNA load, especially for less than 10⁴ IU/ml patients (45.8%~50% vs. 18%~31.4%, $X^2=23.65$, $p=0.02$). This trend was observed in liver fibrosis between HBeAg-positive or negative with high and low DNA load (Fig. 2A). Actually, the proportion of ≥S2 in HBeAg-positive patients with mild or low DNA load and in HBeAg-negative patients with high DNA load were significantly higher, compared with other groups (44.44%~70% vs. 23.1%~27.9%, $X^2=25.5$, $p=0.001$) (Fig. 2B). In the HBeAg-positive patients cohort ($n=200$), overall proportion of SLHI was 50.5% (101/200). Based on different levels of HBV DNA load, the subjects were stratified into five groups, namely >7log (54%, 108/200), 6~7log (15%, 30/200), 4~6log (21%, 42/200) and <4log (10%, 20/200), and liver biopsies with SLHI in the different age groups are depicted in Fig 2C: 39.8% of patients with >7log DNA load had SLHI (43/108), and followed by HBV DNA load decreased, 60~70% of subjects (18/30, 26/42, 14/20) had SLHI ($X^2=11.2$, $P=0.01$). As for HBeAg-negative patients ($n=147$), overall proportion of SLHI was 49.7% (73/147). Based on different levels of HBV DNA load, the subjects were stratified into five groups, namely <2000 (41.5%, 61/147), 2000~4log (28.6%, 42/147), 4~5log (15%, 22/147) and >5log (15%, 22/147). In contrast with HBeAg-positive patients, SLHI rate in HBeAg-negative inactive (HBV DNA <2000 IU/ml) was significantly lower (39.8%), compared with higher HBV DNA load patients (50%~77.3%, $X^2=10.4$, $P=0.02$) (Fig. 2D).

Analysis of Factors Associated with significant liver histological injury (SLHI) in

HBeAg-Positive and -Negative Patients with PNALT

When the presence or absence of SLHI was considered as a binary dependent variable in HBeAg-positive and -negative patients with PNALT, univariate analysis indicated that ALT, AST, HBV DNA, HBeAg, HBsAg and Fibroscan were associated with SLHI in HBeAg-positive and -negative patients with PNALT, respectively (Supplementary Tables 1). However, multiple logistic regression analysis indicated that HBsAg and Fibroscan were associated with SLHI in HBeAg-positive patients with PNALT, and ALT, HBsAg and Fibroscan were the associated factors in HBeAg-negative patients. (Supplementary Tables 1 and 2).

Area Under the Curve (AUC) of some factors Associated with Significant Histological Abnormalities in HBeAg-Positive and -Negative Patients

Receiver operating characteristic (ROC) analysis was used to investigate the predictive ability of LSM and clinical data of SLHI from biopsy reports in patients with CHB (Supplementary Table 2). Among patients with a normal ALT level, the AUC of the ALT level associated with the diagnosis of SLHI was 0.572 and 0.6 in female and male patients, respectively (Female: cut-off value was 20 U/L, 95% confidence interval [CI], 0.48–0.67; sensitivity=60%, specificity=55%; Male: cut-off value was 25 U/L, sensitivity=66%, specificity=54%). Totally, the AUROC of the LSM predicting SLHI risk (0.753, cut-off value 6 Kpa, sensitivity=59.2%, specificity=82.7%) was higher than that of age (0.57, cut-off value 40 years), male (0.48, $P=0.031$), AST (Female: 0.64, cut-off value 22 U/L; Male: 0.63, cut-off value 30 U/L). In the HBeAg-positive patients, AUROC for HBeAg and HBsAg predicting SLHI were 0.66 (cut-off value 104.45 COI) and 0.67 (cut-off value 4 log₁₀ IU/ml), respectively. For HBeAg negative patients, the AUROC for HBsAg was 0.626 with cut-off value 2.86 log₁₀ IU/ml. As for HBV DNA load, the AUROC were 0.63 and 0.65 in HBeAg positive and negative patients, respectively. The cut-off value were less than 7 log₁₀ IU/ml in HBeAg positive patients and more than 3.3 log₁₀ IU/ml.

Among the 347 chronic HBV infection patients with a normal serum ALT,

only 150 patients (43.23%, **Fig3A**) had an ALT level within our cut-off value reference ranges (i.e. 25 U/L for men and 20 U/L for women). Among these 150 patients, only 60 (40%) showed SLHI. A comparison of SLHI between those with ALT levels within the our criteria and those exceeding the our criteria among all 347 patients is shown in Figure 3B. Patients with ALT levels exceeding the our criteria were more likely to have SLHI (57.9% vs. 40%, $p=0.001$, **Fig.3B**). Especially for the HBeAg-positive patients with lower HBV DNA loads and HBeAg-negative patients with higher HBV DNA loads. After revising the ULN of ALT according to our criteria (25 U/L for men and 20 U/L for women), the proportion of SLHI in patients

within ALT criteria (<25 U/L for men and <20 U/L for women) and high HBV DNA loads ($>7\log_{10}$ IU/ml) was significantly lower, compared with patients having lower HBV DNA loads ($\leq 7\log_{10}$ IU/ml) with exceeding ALT criteria (25~50 U/L for men and 20~40 U/L for women) (33.3% vs. 67%, $X^2=17.78$, $P=0.001$, **Fig.3C**). Again, there was significant difference in proportion of SLHI between patients within ALT criteria and less than 2000 IU/ml of HBV DNA loads (32%) and exceeding ALT criteria with more than 2000 IU/ml (70%, $X^2=12.11$, $P=0.006$, **Fig.3D**).

Development of combination indexes estimating significant liver histological injury risk

For totally patients, based on the above single variables, combination indexes were necessary to be constructed to explore whether performance could be improved through logistic analysis. Finally, we developed a novel combination indexes consisting age, LSM, ALT, AST, HBsAg, HBeAg and HBV DNA load according to the classical diagnostic test, and each value was the cut-off value described above. For HBeAg-positive patients, it was means age ≥ 40 yrs, LSM ≥ 6 Kpa, ALT ≥ 25 U/L for male and 20 U/L for female, AST ≥ 25 U/L for male and 22 U/L for female, HBsAg $\geq 4\log_{10}$ IU/mL, HBeAg ≥ 104.45 COI, HBV DNA load $\geq 7\log_{10}$ IU/ml, each item was assigned one point. $\text{Logit}(P) = -1.8 + 0.5 \times \text{Age} - 0.4 \times \text{ALT} + 1.3 \times \text{AST} + 1.1 \times \text{HBeAg} + 1 \times \text{HBsAg} - 0.1 \times \text{HBV DNA} + 1.9 \times \text{LSM}$. The AUROC of the combination indexes was 0.822 with Positive Predictive Value (PPV) 85.92% and negative predictive value (NPV) 70% (95%CI 0.764–0.879), which was higher than any single indicator and the cut-off value was 0.56 (**Fig.4A**). For example, a 43 years HBeAg-positive male patient, who has ALT of 38 U/L and AST 32 U/L, with HBsAg of 4.8 $\log_{10}$ IU/ml, HBeAg of 118 COI and HBV DNA load of 7.2 $\log_{10}$ IU/ml, was 3.3 points base on each item was assigned one point. Therefore, the patient was considered to have SLHI even without biopsy-proven and should be received antiviral therapy.

Among the HBeAg-negative patients, except for the general indicators (age, ALT, AST and LSM), HBsAg and HBV DNA load were adjusted into the. The final formula (each factor was ascribed as one point total that implied a possibility of SLHI presence), as follows: $\text{Logit}(P) = -1.7 - 0.1 \times \text{Age} + 0.2 \times \text{ALT} + 0.7 \times \text{AST} + 1.1 \times \text{HBsAg} + 0.2 \times \text{HBV DNA} + 1.7 \times \text{LSM}$. The AUROC of the combination indexes was 0.818 with PPV 77.65% and NPV 71.69% (95%CI 0.750–0.885), which was higher than any single indicator and the cut-off value was 0.53 (**Fig.4B**).

Discussion

The results of the present study demonstrate that chronic HBV infections with persistently normal ALT (PNALT) levels do not always display no or only minimal histologic injury. The present data indicated that 50.1% (174/347) of patients had significant liver histological injury (SLHI) defined as $\geq G2$ and/or $S2$, which was consistent with three reports from China, USA and India, which reported 37–68.6% [15–17]. These results are notable: as for the invasive nature of liver biopsy, in clinical practice, physicians have primarily used ALT levels as a convenient surrogate marker of liver damage, even if clinical guidelines recommend that liver biopsy was the gold standard for assessing SLHI. Finally, patients with PNALT should not undergo treatment; consequently, these patients are often ignored.

In order to determine the antiviral treatment trigger point and improve survival rates, various treatment guidelines require a certain ALT threshold to rapidly identify patients with CHB at risk of developing SLHI and for treatment decisions. Current ULN for ALT level were set, on average, at 40 U/L in studies conducted over the past 10 years. Such threshold was adopted by EASL [2] and APASL [18] as a crucial criterion for determining treatment initiation for CHB. As for AASLD [5], the ULN of 35 U/L ALT for male and 25 U/L for female adults is recommended when guiding management decisions, which can improve disease outcome. Interestingly, we found that the AUC of the ALT associated with the diagnosis of SLHI was 0.572 for female and 0.6 for male with a cut-off value of 20 U/L and 25 U/L, respectively ($p = 0.04$, $p = 0.013$). Our finding of cut-off value for ALT level was equivalent to that recommended by APASL guidelines that ALT level is divided into high- ($0.5 - 1 \times \text{ULN}$) and low-normal ($\leq 0.5 \times \text{ULN}$) ALT. Base on the new threshold, 43.23% of patients (150/347) was categorized into low-normal ALT and proportion of SLHI was significantly lower, compared to the patients exceeding the our criteria (40% vs. 57.9%, $p = 0.001$).

Except for ALT level, HBeAg status and HBV DNA levels were the important factors to judge the immune status of patients with CHB, which was helpful to evaluate the liver histology, prognosis of the disease and make treatment decision [19]. In our study, with respect to SLHI, the HBV DNA level had reached statistical significance with an AUROC of 0.63 and 0.65 at a level of below 7 $\log_{10}$ IU/ml in HBeAg-positive and over 2000 IU/ml in HBeAg-negative patients. In HBeAg-positive patients, proportion of SLHI in the high normal ALT ($0.5 - 1 \text{ ULN}$) group with lower HBV DNA ($< 7\log_{10}$ IU/ml) has risen to double, compared to the low normal ALT ($< 0.5 \text{ ULN}$) group with high HBV DNA ($\geq 7\log_{10}$ IU/ml) (66.7% vs 33.3%, $p < 0.01$). It is interesting that the latter one was belong to immune tolerant CHB which was defined as HBeAg positive, with HBV DNA $\geq 10^7$ IU/ml in Chinese guidelines, but more rigorous. In the same way, this can be also seen in HBeAg-negative patients with HBV DNA levels at < 2000 IU/ml which are commonly known as “inactive carriers”, and significant liver fibrosis was rare with an estimated rate of 10% [20]. However, our results showed that 37.7% of “inactive carriers” had SLHI totally, among these patients, 70% in patients with high-normal ALT and 32% in low-normal ALT ($P < 0.001$).

In the light of international guidelines such as the AASLD and APASL, as well as Chinese Guidelines (2019), antiviral treatment is not recommended for IT-CHB and inactive carriers [5, 6, 18]. However, more studies had reported using only ALT and HBV DNA levels to define these might mis-classify a fair proportion of gray zone(GZ) patients with significant liver histopathology^[21]. In our study, higher proportion (51.3%) of GZ was reported which including 26.52% of patients with HBeAg positive and HBV DNA < 7log10IU/ml, whereas 24.78% with HBeAg negative and HBV DNA > 2000IU/ml, using conventional ALT criteria with the cut-off of 40 U/L for women and 50U/L for men. The proportion of SLHI were significantly high in the GZ

patients, range from 60 ~ 77.3%. Therefore, we should pay more attention to find GZ and optimize the management. Several researchers suggested to revise the ULN of ALT. Prati et al. had recommended that the ULN for ALT should be decreased to 30 U/L for men and 19 U/L for women [22], Lee et al. also proposed reducing the

ULN of ALT to 35 U/L for men and 25 U/L for women [23]. Similar to our results, the previous studies also had the evidence to revise the ULN of ALT by discovering that high-normal ALT patients had more significant liver histopathology [24]. Gui et al [25] found a significant higher prevalence of liver histopathology in the high-normal ALT group of CHB patients than in the low-normal ALT group (40.0% vs 16.6%, $p < 0.01$), suggesting that a higher proportion of patients with high-normal ALT may have risk of disease progression.

Finally, in order to develop a noninvasive mathematical model to replace liver biopsy to determine SLHI in chronic HBV infection patients, we developed a nomogram that predict SLHI risk according to 7 routine available variables. The predictive value of the nomogram was extremely high with PPV 85.92%, NPV 70% in HBeAg-positive patients and PPV 77.65%, NPV 71.69% in HBeAg-negative patients. Notably, except for ALT, HBV DNA loads discussed above, age > 40yrs, LSM ≥ 6Kpa, HBsAg and HBeAg were rapid and easy to perform in clinical practice. Age is a vital factor affecting the natural course and long term outcomes of patients with chronic HBV infection, and the incidence of adverse events increase with age [26, 27]. In addition, the HBeAg-positive phase of CHB is usually associated with viral repli

cation and hepatitis activity [28, 29]. Persistent presence of HBeAg has been demonstrated to be associated with a higher risk of fibrosis progression and cirrhosis development. Therefore, it is important for clinicians to monitoring disease progression in GZ CHB patients with older age and high-normal ALT who might have higher risk of advanced disease

There were several limitations that affected the interpretation of our findings. This study was a retrospective cross-sectional study that could not exclude selection bias of patients. Moreover, the number of patients aged 50 years was relatively small, and follow-up of patients after liver biopsy was insufficient. Findings of this study could not be generally applied to the genotype other than genotype B.

Declarations

Author Contribution

Ren Shan and Chen Xinyue contributed to the conception of the study; Wang Kefei and Zheng Yanhong contributed to the collection of the clinical data; Ren Shan and Lu Jufeng contributed significantly to analysis and manuscript preparation; Ren Shan and Ma Lina performed the data analyses and wrote the manuscript; Chen Xinyue and Zheng Sujun helped perform the analysis with constructive discussions.

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Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon request.

Consent to Participate (Ethics)

The study protocol was permitted by the ethics committee of Beijing Youan hospital, Capital Medical, China, and the procedures were in accordance with the Helsinki declaration of 1975, as revised in 1983(The Code of Ethic Approval:[2020]089)

Consent for publication(Ethics)

Ethics Committee approval was obtained from the Institutional Ethics Committee of Capital Medical University Beijing Youan hospital to the commencement of the study.

Competing interests

The authors declared no conflict of interests.

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Tables

Table 1. Baseline characteristics of enrolled patients.

Clinical characteristics	Total (n=347)	HBeAg-positive patients (n=200,57.64%)			HBeAg-negative patients (n=147,42.36%)		
		Group A (n=108)	Group B (n=72)	Group C (n=20)	Group D (n=86)	Group E (n=61)	Value
Age(Year)	38.91±9.62	35.02±7.30	37.31±10.10	38.80±7.83	43.40±10.36	41.43±9.04	F=12.26,p<0.001
Male n(%)	210(60.5%)	63(58.3%)	49(68.1%)	16(80%)	23(52.3%)	59(62.3%)	χ ² =6.80,p=0.14
BMI (kg/m ²)	22.82±4.23	21.42±4.23	23.1±3.92	23.19±2.10	22.32±5.12	20.11±4.12	F=0.28,p=0.34
ALT (U/L)	26.34±9.82	28.64±9.38	28.40±9.41	26.71±10.69	24.68±9.90	22.07±9.07	F=7.30p<0.001
AST(U/L)	23.21±11.2	22.10±110.12	27.64±17.0	23.86±7.31	23.14±8.81	19.80±6.31	F=5.70,p=0.003
PLT (10 ⁹ /L)	206±78.82	248±96.00	218±86.00	211±72.00	185±62.00	209±89.00	F=0.83,p=0.09
ALB (g/L)	43.0 ±4.20	45.0±5.17	43.0±3.65	43.0 ±4.81	44.0±3.78	41.32±5.16	F=0.78,p=0.64
ALP (U/L)	70.0±29.0	72.0±28.0	73.2±23.4	69.0±32.1	76.0±21.8	71.0±28.0	F=0.66,p=0.61
GGT (U/L)	22.0±27.0	20.0±13.0	24.0±18.0	23.0±19.8	20.9±14.8	23.4±17.0	F=0.59,p=0.56
Genotype C (n,%)	266 (76.1%)	82 (75.92%)	54(75)	12(60%)	34(77.27%)	84(81.53%)	χ ² =3.46,p=0.09
HBVDNA(log10IU/mL)	5.40±2.25	8.14±0.60	5.60±0.91	2.81±1.00	4.40±1.03	2.57±0.56	F=655.4,p<0.001
HBsAg(log10 IU/ml)	3.55±0.94;	4.07±0.77	3.62±0.78	3.44±0.55	3.23±0.65	2.86±1.22	F=22.78,p<0.001
HBeAg (COI/ml)	565.49±826	1386.68±703.65	599.03±903.1	175.57±378	0.13±0.15	0.11±0.04	F=92.51,p<0.001
Fibroscan:LSM(Kpa)	6.14±4.02	6.10±6.35	6.44±2.75	6.34±2.69	6.46±2.38	5.31±1.04	F=13.82,p=0.85

*BMI:Body mass index;PLT:Platelet counts;ALB:Albumin;ALP:Alkaline phosphatase;GGT: g glutamyltransferase;SLHI:significant liver histological injury,defined as inflammation grade≥2 (≥G2) and/or fibrosis stage≥2(≥F2).

Table 2. Distribution of liver necroinflammation and fibrosis in different immune states groups (%)

liver histopathology	Total (n=347)	HBeAg-positive patients (n=200,57.64%)			HBeAg-negative patients (n=147,42.36%)		values
Inflammation n (%)		Group A (n=108)	Group B (n=72)	Group C (n=20)	Group D (n=86)	Group E (n=61)	χ ² =23.65,p=0.02
G1	233(67.1%)	75(69.4%)	39(54.7%)	10(50%)	59(68.6%)	50(82%)	
G2	101(29.1%)	31(28.1%)	27(37.5%)	8(40%)	24(27.9%)	11(18%)	
G3	12(3.5%)	2(1.9%)	6(8.3%)	2(10%)	2(2.3%)	0(0)	
G4	1(0.3%)	0(0)	0(0)	0(0.7%)	1(1.2%)	0(0)	
≥G2	114(32.9%)	33(30.6%)	33(45.8%)	10(50%)	27(31.4%)	11(18%)	χ ² =14.58,p=0.006
Fibrosis n (%)							χ ² =36.73,p=0.002
S0	26(7.5%)	9(8.3%)	4(5.6%)	0(0)	8(9.3%)	5(8.2%)	
S1	193(55.6%)	74(68.5%)	36(50%)	6(30%)	38(44.2%)	39(63.9%)	
S2	84(24.2%)	16(14.8%)	19(26.4%)	10(50%)	23(26.7%)	16(26.2%)	
S3	37(10.7%)	9(8.3)	10(13.9%)	3(15%)	14(16.3%)	1(1.6%)	
S4	7(2%)	0(0)	3(4.3%)	1(5%)	3(3.5%)	0(0)	
≥S2	128(36.9%)	25(23.1%)	32(44.4%)	14(70%)	40(46.5%)	17(27.9%)	χ ² =25.5,p=0.001
SLHI n(%)	174(50.1%)	43(39.8%)	44(61.1%)	14(70%)	50(58.1%)	23(37.7%)	χ ² =17.20,p=0.002

Figures

Figure 1

Flow chart of study design(NAFLD: non alcoholic fatty liver disease; HCV:hepatitis C virus;HDV:hepatitis D virus;HIV:human immunodeficiency virus;PNALT:Persistent normal ALT; ULN:Male $\leq$ 50 U/L; Female $\leq$ 40 U/L)

Figure 2

Distribution of liver inflammation and fibrosis in different immune states groups (%). (A) Significant differences in liver inflammation were observed in all patients. (B)Significant differences in liver fibrosis were observed in all patients. (C)various HBV DNA load in HBeAg-positive patient: 39.8% of patients with>7log DNA load had SLHI, and followed by HBV DNA load decreased, SLHI rate increased. (D)various HBV DNA load in HBeAg-negative patient: 37.7% of patients with<2000IU/ml DNA load had SLHI, and followed by HBV DNA load increased, SLHI rate increased.

Figure 3

Comparison of proportion of SLHI among patients with normal ALT. (A) Among totally patients with a normal serum ALT,only 43.23% of them within our cut-off value reference ranges of ALT level. (B)Significant differences in SLHI were observed in patients within our criteria and exceeding our criteria of ALT level. (C)In HBeAg-positive patient: 67% of patients with≤7log IU/ml DNA loads and exceeding our criteria of ALT level had high SLHI. (D)In HBeAg-negative patient: 70% of patients with>2000IU/ml DNA loads and exceeding our criteria of ALT level had high SLHI.

Figure 4

Analysis of AUROC for combination indexes as indicators for SLHI. (A) In HBeAg-positive patients: the AUROC was 0.822 ($P < 0.001$); (B) In HBeAg-negative patients: the AUROC was 0.818 ($P < 0.001$). AUROC, area under the receiving operator characteristic curve.

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